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Review

Role of autophagy in *Caenorhabditis elegans*

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ABSTRACT

Autophagy is an evolutionarily conserved intracellular catabolic system. During *Caenorhabditis elegans* development, autophagy plays an important role in many physiological processes, including survival under starvation conditions, modulation of life span, and regulation of necrotic cell death caused by toxic ion-channel variants. Recently, it has been demonstrated that during embryogenesis, basal levels of autophagy selectively remove a group of proteins in somatic cells, including the aggregate-prone components of germline P granules. Degradation of these protein aggregates provides a genetic model to identify essential autophagy components and also to elucidate how the autophagic machinery selectively recognizes and degrades specific targets during animal development.

1. A brief historical background of autophagy research

Autophagy was first described in the late 1950s and early 1960s starting with the finding of partially degraded but still recognizable cytoplasmic elements inside membrane-bound bodies in kidney and liver cells [1]. The realization that these bodies are in fact lysosomes breaking down self-derived cytoplasmic material led deDuve and Wattiaux to introduce the name and concept of autophagy as a general mechanism for bulk lysosomal degradation of cytoplasmic components in eukaryotic cells [2]. Autophagy has now been dissected into several distinct steps, including the formation of a cup-shaped membrane sac (also known as the phagophore or the isolation membrane) that engulfs a portion of cytoplasm; elongation and complete closure of the membranes to form the autophagosome; transport and fusion of the autophagosome with the lysosome to form the autolysosome; and finally degradation of the enclosed cytoplasmic contents [3,4]. Mounting evidence has demonstrated that autophagy is a fundamentally important catabolic process in eukaryotic cells and participates in the regulation of key physiological, developmental and pathological events [5].

Until the early 1990s, autophagy was studied by the methods of classical cell biology and biochemistry, including electron microscopy, cell fractionation, and measurement of labeled intracellular proteins [6,7]. The classical observations offer valuable reference points and create a stable foundation for the evaluation of new data in many traditional model systems of autophagy. The new era of autophagy research started with the identification of a set of conserved proteins (Atg proteins) in yeast that are essential for starvation-induced autophagy and the autophagy-related cytoplasm to vacuole targeting (Cvt) pathway [8,9]. Almost all known Atg proteins associate with the pre-autophagosomal structure (PAS), which is thought to be the site of autophagosome assembly in yeast [3]. These Atg proteins act at distinct steps of autophagosome biogenesis. The Atg1 serine/threonine protein kinase complex, consisting of Atg1 and Atg13, integrates upstream TOR (target of rapamycin) signaling with the autophagy machinery. The Vps34/Atg6 class III phosphatidylinositol 3-kinase complex is required for the nucleation and assembly of the initial isolation membrane. The expansion and completion of the autophagosome is controlled by the two ubiquitin-like conjugation systems, in which Atg8 is conjugated to phosphatidylethanolamine (PE) through the sequential action of a cysteine proteinase, Atg4, an E1-like activating enzyme, Atg7 and an E2-like conjugating enzyme, Atg3. Atg12 is conjugated to Atg5 through the action of Atg7 and an E2-like enzyme, Atg10. The Atg12/Atg5 conjugates further associate with Atg16 to form a multimeric complex mediated by Atg16 homo-oligomerization. Recycling of Atg9 membrane protein between autophagic structures and other organelles depends on the Atg1/Atg13 complex, Atg2 and Atg18 [3,4]. The mechanism by which these proteins act collaboratively to form autophagosomes still remains largely unknown.

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2. Conservation of yeast autophagy genes in *C. elegans*

Caenorhabditis elegans develops in ~3 days through an embryonic stage and four larval stages separated by molting (L1, L2, L3 and L4) into a reproductively mature adult, which will live another 2–3 weeks in favorable conditions (Fig. 1) [10]. *C. elegans* is a relatively simple animal (959 somatic cells in the hermaphrodite), transparent with an invariant cell lineage, self-fertilizing and easy to culture and has been a major player in many biological discoveries, including programmed cell death, RNA interference and micro RNA. *C. elegans*, however, is not easily accessible by the main methods of traditional autophagy studies such as enzyme cytochemistry, cell fractionation and electron microscopy [11]. In spite of these difficulties, the wealth of knowledge about *C. elegans* developmental biology, as well as powerful genetic tools such as forward genetic screens for mutants, makes *C. elegans* an appealing model system to study autophagy.

Conservation of the yeast Atg proteins at both the sequence and functional levels has greatly facilitated the study of autophagy in other organisms. *C. elegans* has a single ortholog of most yeast Atg proteins, except that there are two homologs of *atg-4, atg-8* and *atg-16* (Table 1). It remains to be determined whether both homologs are functional, act in different tissues or developmental stages, or have distinct functions in different autophagy-mediated processes.

3. Monitoring autophagy in *C. elegans*

3.1. Transmission electron microscopy

Traditional transmission electron microscopy (TEM) was the first and for a long time the main tool to study autophagy. Although methods of light microscopic visualization of GFP tagged Atg proteins have become possible and more favored, the analysis by TEM remains the ultimate reference point when the validity of the light microscopic methods comes into question. In addition, ultrastructural details of autophagic elements and the cytoplasmic components involved in or related to autophagy can be observed only by highly resolution images provided by TEM. The identification and detailed description of autophagic elements in *C. elegans* by TEM has been described previously [11,12]. Although fragmentary, TEM data supports the view that autophagy in *C. elegans* appears in various developmental and physiological events and in many cell types.

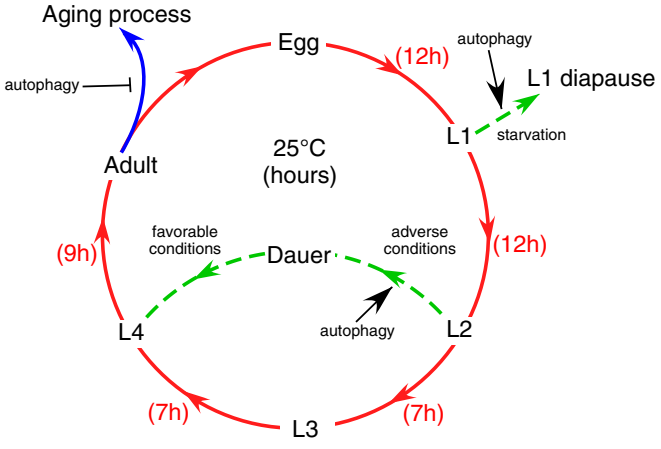

Fig. 1. The life cycle of *C. elegans* at 25 °C.

Table 1
C. elegans homologs of yeast autophagy genes.

Yeast gene	Homolog in *C. elegans*
tor1, tor2	*let-363*
atg1	*unc-51*
atg2	M03A8.2
atg3	*atg-3*
atg4	Y87G2A.3, ZK792.8
atg5	Y71G12B.12
atg6	*bec-1*
atg7	*atg-7*
atg8	*lgg-1, lgg-2*
atg9	T22H9.2
atg10	*atg-10*
atg12	*lgg-3*
atg13	*epg-1*
atg16	F02E8.5, K06A1.5
atg18	*atg-18*
vps34	*vps-34*

3.2. The LGG-1/Atg8 reporter

The Atg8/LC3 (the mammalian Atg8 homolog) reporter is the most commonly used tool to detect autophagy in yeast and mammalian cells [13]. The PE-conjugated form of Atg8/LC3 is localized to both the inner and outer membrane layers of the isolation membrane. Following autophagosome maturation, Atg8 on the outer membrane is cleaved off by Atg4 and recycled, while Atg8 on the inner membrane is transported into the lysosome for degradation. Thus, Atg8/LC3 associates with autophagosomal membranes during autophagy. The *C. elegans* Atg8 reporter, GFP::LGG-1, also forms punctate structures in various cell types, including pharyngeal muscles and seam cells [14,15]. The difficulties of performing EM analysis in *C. elegans* make researchers heavily, and in most cases solely, reliant on the LGG-1 reporter to monitor autophagy during animal development. LGG-1 puncta, however, do not always represent autophagic structures. GFP::LGG-1 incorporates into protein aggregates in autophagy mutants [16]. Therefore, LGG-1 localization should be carefully interpreted and the occurrence of autophagy in *C. elegans* should be examined by additional methods, including electron microscopy and degradation of well-characterized preferential autophagy targets.

4. Role of autophagy in maintaining survival of animals under starvation conditions

4.1. Survival of L1 larvae in the absence of food

In the absence of food, newly hatched larvae fail to initiate larval development and arrest at the L1 diapause, which maintains viability for about 1–2 weeks (Fig. 1). Inhibition of autophagy decreases survival of wild type worms during L1 starvation [15]. Starvation activates autophagy in pharyngeal muscles, as measured by the formation of GFP-LGG-1 punctate structures, through the muscarinic acetylcholine signaling pathway (via the G-protein-coupled acetylcholine receptor → Gqα → nPKC → MAPK) [15,17]. DAPK-1, the *C. elegans* ortholog of death-associated protein kinase, appears to act downstream of muscarinic acetylcholine receptor signaling to regulate autophagy in pharyngeal muscles [15]. Loss of function of *dapk-1* reduces starvation-induced autophagy. In animals mutant for *gpb-2* (encoding a G-protein β subunit mutants), muscarinic acetylcholine receptor signaling is overactive, and starvation induces excess autophagy, subsequently leading to death [15]. Reduced activity of *bec-1* (encoding the *C. elegans* Atg6/Beclin 1 homolog) or *dapk-1* partially rescues the death of *gbp-2* mutant

animals during starvation. Thus, physiological levels of autophagy promote survival, whereas insufficient or excessive levels of autophagy contribute to death during L1 starvation.

4.2. Dauer formation

When early larvae are exposed to harsh environmental conditions, including high population density, limited food supply or high temperature, they develop to a specialized third larval stage called the dauer diapause, which exhibits morphological and behavioral characteristics distinct from normal L3 larvae, including formation of a special cuticle with alae (ridges), lack of pharyngeal pumping, increased fat storage, resistance to detergent treatment and long-term survival (up to several months) [18]. Upon return to favorable environments, dauer larvae recover and develop into reproductive adults (Fig. 1). During dauer formation, GFP::LGG-1 forms punctate structures in seam cells, which play essential roles in dauer morphogenesis, such as formation of dauer alae [14]. Reducing the activity of *daf-2*, which encodes the insulin/IGF-1 receptor, causes animals to enter dauer development even in favorable conditions. However, simultaneous depletion of autophagy activity in *daf-2* mutants results in a defect in dauer formation and animals die within a few days [14]. Thus, autophagy plays a key role in maintaining viability during starvation conditions in *C. elegans*.

5. Regulation of the adult aging process by autophagy

During adulthood, *C. elegans* somatic cells, which are post-mitotic, undergo progressive decline in pharyngeal pumping and body movement and also experience cell and tissue deterioration. Adult worms also accumulate damaged proteins and organelles. The aging process is regulated by several mechanisms, including dietary restriction, insulin/IGF-1 signaling and the steroid hormone system [19]. Autophagy is a key catabolic process in removing damaged proteins and/or organelles, accumulation of which would be harmful to the animal. Not surprisingly, inactivation of autophagy genes accelerates aging and shortens the life span [20]. Feeding-defective mutants, the phenotype of which mimics dietary restriction, exhibit more GFP-positive punctate areas in seam cells than wild type worms, indicating elevated autophagy activity [20–22]. The small GTPase RAB-10 and the FOXA transcription factor PHA-4 are involved in the regulation of autophagy in response to dietary restriction [22]. Loss of function of *rab-10* increases the number of LGG-1 foci in larvae and extends life span in an autophagy-dependent manner. The increased autophagy levels in *rab-10* and feeding-defective mutants require the activity of *pha-4* [22]. In addition to extension of lifespan induced by dietary restriction, autophagy is required for longevity conferred by reduced activity of insulin/IGF-1 signaling, reduced TOR kinase activity, lowered mitochondrial respiration, and increased dose of *sir-2.1* (encoding the *C. elegans* NAD^+-dependent protein deacetylase) [14,20–24]. Thus, different longevity pathways converge on autophagy genes to slow down aging and lengthen lifespan.

6. Role of autophagy in cell death

6.1. Necrosis

Necrotic cell death, which is a common feature of various human neurodegenerative disorders such as Alzheimer's, Parkinson's and Huntington's diseases, is characterized by dramatic membrane infolding, degradation of cytoplasm contents, and vacuole formation. In *C. elegans*, formation of GFP::LGG-1 punctate structures is induced early during the necrotic cell death of a subset of neurons caused by gain-of-function mutations in genes encoding ion-channel subunits such as DGE-1 and MEC-4. Degeneration of these neurons is partially suppressed by inactivation of *unc-51*, *bec-1* and *lgg-1* [25,26], indicating that autophagy contributes to necrotic-like degeneration caused by toxic ion-channel variants.

6.2. Apoptosis

During *C. elegans* development, 131 somatic cells undergo apoptotic cell death and the resulting cell corpses are engulfed and degraded by neighboring cells through the lysosome system [27]. Apoptotic cell death in *C. elegans* is inhibited by the homolog of the anti-apoptotic protein Bcl-2, CED-9. In mammalian cells, Bcl-2 inhibits autophagy by binding Beclin 1, thus preventing formation of the Beclin 1/VPS34 complex that is essential for the autophagosome nucleation step [28]. In *C. elegans*, BEC-1 forms a complex with VPS-34 and also with CED-9 [29]. Knockdown of *bec-1* activity causes a significantly higher number of apoptotic cell corpses than in wild type animals, indicating that inactivation of *bec-1* triggers apoptotic cell death [29]. Recent studies show that autophagy is involved in generating energy-dependent engulfment signals, such as exposure of the "eat-me" signal phosphatidylserine (PS) on the outer membrane surface of apoptotic cells, thus contributing to the removal of apoptotic cell corpses [30,31]. This raises the possibility that the excess apoptotic cell corpses in *bec-1* mutants could also result from the delay in clearance of cell corpses.

7. Degradation of polyglutamine repeat-containing proteins by autophagy

Expansion of polyglutamine (polyQ) repeats in various proteins has been causally linked to a number of neurodegenerative diseases, including Huntington's disease and spinocerebellar ataxia (SCA). In *C. elegans*, animals expressing a polyQ fragment containing 40 glutamine residues in muscle cells develop increasing numbers of cytoplasmic aggregates from the late larval stage onwards and show locomotion defects [32]. Loss of autophagy activity causes an earlier onset of formation and an increased number of polyQ aggregates, as well as exacerbating the polyQ-induced muscle dysfunction [33]. Loss of autophagy activity also results in formation of aggregates of non-pathogenic polyQ (33Q) fragments [34]. Degeneration of the ASH sensory neuron caused by a polyQ tract of 150 residues is accelerated by inactivation of autophagy genes [33]. These *C. elegans* polyQ-induced toxicity models demonstrate that autophagy attenuates cell toxicity caused by polyQ aggregates.

8. Degradation of aggregate-prone germline P granule components in somatic cells by autophagy

Generation of germline cells in *C. elegans* involves four sequential unequal divisions in which a blastomere generates one somatic founder cell and one germline blastomere, P1, P2, P3 and P4 (Fig. 2A). The primordial germ cell P4 then divides equally at the ~100-cell embryonic stage, giving rise to two germ precursor cells, Z2 and Z3, which proliferate throughout larval development to produce germ cells [35]. During the generation of germline cells, germ P granules, which form a specialized germ plasma thought to carry germline determinants, are exclusively segregated into the germline lineage (Fig. 2B and C). P granules continue to be expressed in all descendants of P4 with the exception of mature sperm [35]. Several mechanisms are known to mediate the asymmetric partitioning of P granules into germline cells, including migration with the central cytoplasm flow to the posterior end

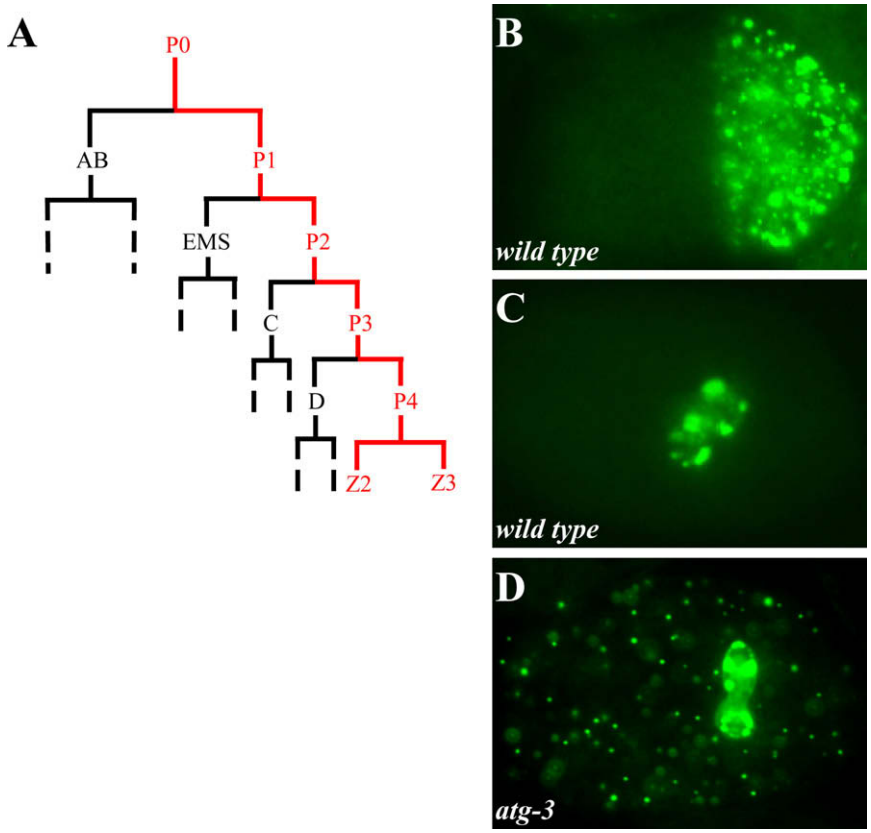

Fig. 2. Segregation of P granules into germ blastomeres. (A) Generation of germ precursor cells during embryogenesis. Germline cells are shown in red; somatic cells are shown in black. Descendants of the somatic founder cells (AB, EMS, C and D) are indicated by dashed black lines. (B) Localization of P granules, detected by anti-PGL-3 antibody, in the germline blastomere P1 at the 2-cell stage. (C) P granules are restricted to germ precursor cells Z2 and Z3. (D) Presence of PGL-3-positive granules in somatic cells in an *atg-3* mutant embryo. The strongly labeled cells are Z2 and Z3.

(in P0 and P1 cells), association with the nucleus and deposition in the region destined to be inherited by germline cells at nuclear membrane breakdown (in P2 and P3 cells), and degradation of P granules that remain in somatic blastomeres [36].

P granules contain constitutive components that associate with P granules during all developmental stages, including the GLH-1 RNA helicase and RGG box-containing RNA binding proteins PGL-1 and PGL-3, and also transient components that interact with P granules only during early embryogenesis, including the CCCH-type zinc finger proteins PIE-1, MEX-1 and POS-1 [35]. During early embryogenesis, CCCH finger proteins that remain in somatic blastomeres are removed by the cullin-dependent degradation system [37], while PGL-1 and PGL-3 are degraded by autophagy. In autophagy mutants, PGL-1 and PGL-3 accumulate into aggregates, termed PGL granules, in somatic cells (Fig. 2D) [38].

9. SEPA-1 functions as an adaptor protein in mediating the autophagic degradation of PGL granules

Formation of PGL granules in autophagy mutants requires the novel coiled-coil domain-containing protein SEPA-1 [38]. PGL-1 and PGL-3 are diffusely localized in the cytoplasm in *sepa-1* mutants. SEPA-1 itself forms aggregates and is also selectively removed by autophagy during embryogenesis, so that SEPA-1 aggregates are present only in early embryos [38]. In autophagy mutants, large numbers of SEPA-1 aggregates persist in late stage embryos and larvae. SEPA-1 aggregates colocalize with PGL granules in autophagy mutants. Accumulation of PGL-1 into PGL granules also depends on PGL-3. SEPA-1 directly interacts with PGL-3 [38]. PGL-1 associates with PGL-3, but does not interact with SEPA-1 [38,39]. Therefore, SEPA-1 is able to form oligomeric structures that further recruit PGL-3 and PGL-1 to form PGL granules in autophagy mutants.

SEPA-1 is also required for degradation of PGL granule components by autophagy [38]. Degradation of PGL-1 also depends on PGL-3. Formation of SEPA-1 aggregates and their degradation by autophagy is independent of PGL-3 and PGL-1 [38]. Similarly, PGL-1 is dispensable for accumulation and degradation of PGL-3. SEPA-1 interacts directly with LGG-1 and binding of LGG-1 may trigger a cascade of events that lead to the formation of autophagosomes surrounding PGL granules [38]. Thus, SEPA-1 functions as an adaptor that links PGL granules to the autophagy machinery (Fig. 3).

The composition, distribution and organization of PGL granules in somatic cells in autophagy mutants are distinct from that of P granules in germline cells. PGL aggregates are dispersed in the cytoplasm, while P granules associate with the outer surface of the nuclear envelope at all stages of germline development except oogenesis and early embryogenesis [35,38]. P granules contain transient components and GLH family RNA helicases that are absent in PGL granules. SEPA-1 is an integral component of PGL granules, but is not found in germline cells [38]. PGL-3 is required for accumulation of PGL-1 into PGL granules, but is dispensable for its association with P granules [38,39]. Furthermore, association of PGL-1 and PGL-3 with P granules depends on GLH-1 [40]. Thus, the assembly of somatic PGL granules is different from that of germline P granules.

10. Physiological function of autophagic degradation of PGL granules in somatic cells

The development of *C. elegans* embryos from the one-cell stage to the end of embryogenesis (558 cells) mainly relies on

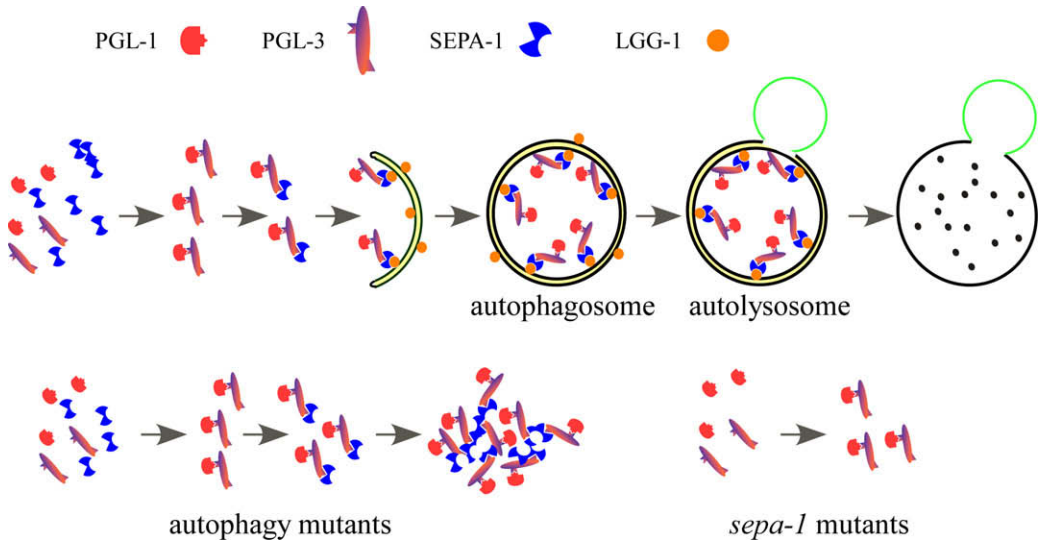

Fig. 3. SEPA-1 functions as an adaptor protein in linking PGL granules to the autophagic machinery. Model for the degradation of somatic PGL granule components by autophagy. Binding of SEPA-1 to the autophagy protein LGG-1 may trigger a cascade of events that leads to autophagosome formation. SEPA-1 acts as an adaptor protein in mediating the degradation of PGL-3 by autophagy. Degradation of PGL-1 appears to be mediated through its interaction with PGL-3. A lysosome is shown in green and degraded proteins are indicated by black dots. In autophagy mutants, PGL-1 and PGL-3 accumulate with SEPA-1 into aggregates. PGL-1 and PGL-3 are diffusely localized in the cytoplasm in *sepa-1* mutants.

the degradation of maternally-loaded yolk vesicles. PGL granules that remain in somatic cells during early embryonic divisions could also provide a nutrient resource for embryogenesis. Moreover, formation of PGL aggregates could be detrimental to embryo development. The hatching defect in autophagy mutants is significantly suppressed by *sepa-1* mutation [41].

Autophagy appears to be generally required for the transition from oocyte to embryo. In mice, autophagy activity is triggered by fertilization and up-regulated in early embryos. Autophagy-defective oocytes arrest at the 4- to 8-cell stage [42]. Autophagy could be involved in degradation of maternal proteins to provide energy and/or in removal of maternally-loaded macromolecules or obsolete materials that would be harmful for later development.

11. Similarity between SEPA-1 and p62

The role of SEPA-1 in mediating the formation and degradation of PGL granules resembles that of p62/sequestosome 1 (SQSTM1) in the formation and degradation of polyubiquitated protein aggregates in mammalian cells. p62 is able to polymerize via the N-terminal PB1 domain to form protein aggregates. p62 is also degraded by autophagy and inhibition of autophagy leads to an increase in the size and number of p62 aggregates [43]. p62 binds to polyubiquitinated proteins via its C-terminal ubiquitin-associated (UBA) domain and is required for the formation of polyubiquitinated protein aggregates [43,44]. p62 interacts directly with Atg8 to facilitate degradation of polyubiquitinated proteins [45]. Unlike SEPA-1, which appears to be specifically involved in degradation of PGL granules, p62 non-selectively binds to polyubiquitinated protein aggregates.

Degradation of PGL granules and polyubiquitinated protein aggregates is mechanistically similar to the Cvt pathway in yeast, which involves transport of the vacuolar protein aminopeptidase 1 (Ape1) from the cytoplasm to the vacuole [4]. The precursor form of Ape1 oligomerizes to form a dodecamer, which further binds to the receptor Atg19 and assembles into a higher order Cvt complex. The Cvt complex is transported to the PAS, and enclosed by double membrane vesicles which subsequently fuse with the vacuole [4]. Atg19 links the Cvt complex to the vesicle formation machinery via interaction with Atg8 [46]. Unlike SEPA-1 and p62, Atg19 is not required for the formation of prApe1 complex and degradation of Atg19 depends on prApe1. Atg19 is uniformly distributed in the cytoplasm in *ape1* deletion cells [46]. Nevertheless, a common theme emerging from these studies is that a group of proteins function as bridging molecules to link cargo proteins with Atg8. Binding of Atg8 may induce the formation of autophagic structures surrounding the cargo-receptor complex.

12. Identifying novel components of the autophagy pathway in *C. elegans*

Degradation of PGL granules by autophagy provides a genetic model to identify essential components of the autophagy pathway. A novel gene, *epg-1*, was isolated in a screen for mutants with defective degradation of somatic PGL granules. Loss of function of *epg-1* causes defects in many autophagy-regulated processes [16]. The N-terminus of EPG-1 shows high similarity to mammalian Atg13. In yeast and mammalian cells, Atg13 binds to Atg1 and acts at several steps of autophagosome formation [3]. EPG-1 directly interacts with UNC-51, indicating that EPG-1 may be highly divergent to ATG-13 [16].

In addition to ATG-13, UNC-51 interacts with UNC-14 and VAB-8 in regulating axonal guidance [47–49]. For example, *unc-51* and *unc-14* are required for circumferential axonal growth over the lateral hypodermis and longitudinal axonal elongation along the nerve cords [47,48]. *epg-1* mutants have normal locomotion and show no obvious defect in axon guidance [16]. Thus, UNC-51 functions outside the autophagy pathway by interacting with distinct factors.

13. Conclusion and future directions

The molecular understanding of autophagy has come almost exclusively from yeast genetic studies. In higher eukaryotes, autophagy seems to involve much more complex membrane dynamics. No defined PAS has been identified and several isolation membranes can form simultaneously [5]. Recently, it was found that at least some autophagosomes are generated from omegasomes, which are phosphatidylinositol 3-phosphate (PI(3)P) enriched membrane compartments of the endoplasmic reticulum

(ER) [50,51]. Autophagosomes are transported to the perinuclear region, where lysosomes are enriched. Lysosomes are much smaller than vacuoles (the yeast lysosome counterpart) and their size is comparable to that of autophagosomes. Recent studies showed that the nascent autophagosome undergoes a stepwise maturation step, including fusion with endosomes and multivesicular bodies, before fusion with lysosomes [52–54]. However, very little is known about higher eukaryote-specific autophagy components that control autophagosome formation, modulate autophagy activity or regulate the convergence of autophagosomes with the endocytic pathway. The introduction of *C. elegans* as a model organism is an outstanding success story of modern biology. Autophagy has already been shown to play an important role in many physiological processes during *C. elegans* development. Identification of a group of preferential autophagy targets in *C. elegans* will allow us to perform genetic screens to identify autophagy components specific to higher eukaryotes and further assemble these genes into genetic pathways. These studies will help us to understand the molecular mechanisms of induction, expansion and maturation of autophagosomes and also of selective autophagic degradation of protein aggregates associated with various neurodegenerative diseases in mammals.

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References

[1] Ericsson, J.L. (1969) Mechanism of cellular autophagy (Dingle, J.T. and Fell, H.B., Eds.), Lysosomes in Biology and Pathology, vol. 2, pp. 345–395, North Holland, Amsterdam-London.
[2] de Duve, C. and Wattiaux, R. (1966) Functions of lysosomes. Annu. Rev. Physiol. 28, 435–492.
[3] Nakatogawa, H., Suzuki, K., Kamada, Y. and Ohsumi, Y. (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat. Rev. Mol. Cell Biol. 10, 458–467.
[4] Xie, Z. and Klionsky, D.J. (2007) Autophagosome formation: core machinery and adaptations. Nat. Cell Biol. 9, 1102–1109.
[5] Mizushima, N. (2007) Autophagy: process and function. Genes Dev. 21, 2861–2873.
[6] Glaumann, H. and Ballard, F.J. (1987) Lysosomes their Role in Protein Breakdown, Academic Press.
[7] Seglen, P.O. and Bohley, P. (1992) Autophagy and other vacuolar protein degradation mechanisms. Experientia 48, 158–172.
[8] Tsukada, M. and Ohsumi, Y. (1993) Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. FEBS Lett. 333, 169–174.
[9] Harding, T.M., Morano, K.A., Scott, S.V. and Klionsky, D.J. (1995) Isolation and characterization of yeast mutants in the cytoplasm to vacuole protein targeting pathway. J. Cell Biol. 131, 591–602.
[10] Brenner, S. (1974) The genetics of *Caenorhabditis elegans*. Genetics 77, 71–94.
[11] Kovács, A.L., Vellai, T. and Müller, F. (2004) Autophagy in *Caenorhabditis elegans* in: Autophagy, Eurekah.com, Landes Bioscience (Klionsky, D.J., Ed.), pp. 217–223, Austin, Georgetown, Texas, USA.
[12] Sigmond, T., Feher, J., Baksa, A., Pasti, G., Palfia, Z., Takacs-Vellai, K., Kovacs, J., Vellai, T. and Kovacs, A.L. (2008) Qualitative and quantitative characterization of autophagy in *Caenorhabditis elegans* by electron microscopy. Methods Enzymol. 451, 467–491.
[13] Mizushima, N. (2004) Methods for monitoring autophagy. Int. J. Biochem. Cell Biol. 36, 2491–2502.
[14] Melendez, A., Talloczy, Z., Seaman, M., Eskelinen, E.L., Hall, D.H. and Levine, B. (2003) Autophagy genes are essential for dauer development and life-span extension in *C. Elegans*. Science 301, 1387–1391.
[15] Kang, C., You, Y.J. and Avery, L. (2007) Dual roles of autophagy in the survival of *Caenorhabditis elegans* during starvation. Genes Dev. 21, 2161–2171.
[16] Tian, E., Wang, F.X., Han, J.H. and Zhang, H. (2009) *Epg-1* functions in the autophagy pathway and may encode a highly divergent Atg13 homolog in *C. elegans*. Autophagy 5, 608–615.
[17] You, Y.J., Kim, J., Cobb, M. and Avery, L. (2006) Starvation activates MAP kinase through the muscarinic acetylcholine pathway in *Caenorhabditis elegans* pharynx. Cell Metab. 3, 237–245.
[18] Hu, J.P. (2007) Dauer. Wormbook August 08, 1–19.
[19] Kenyon, C. (2005) The plasticity of aging: insights from long-lived mutants. Cell 120, 449–460.
[20] Tóth, M.L., Sigmond, T., Borsos, E., Barna, J., Erdélyi, P., Takács-Vellai, K., Orosz, L., Kovács, A.L., Csikós, G., Sass, M. and Vellai, T. (2008) Longevity pathways converge on autophagy genes to regulate life span in *Caenorhabditis elegans*. Autophagy 4, 330–338.
[21] Mörck, C. and Pilon, M. (2006) *C. elegans* feeding defective mutants have shorter body lengths and increased autophagy. BMC Dev. Biol. 6, 39.
[22] Hansen, M., Chandra, A., Mitic, L.L., Onken, B., Driscoll, M. and Kenyon, C. (2008) A role for autophagy in the extension of lifespan by dietary restriction in *C. elegans*. PLoS Genet. 4, e24.
[23] Jia, K. and Levine, B. (2007) Autophagy is required for dietary restriction-mediated life span extension in *C. elegans*. Autophagy 3, 597–599.
[24] Hars, E.S., Qi, H., Ryazanov, A.G., Jin, S., Cai, L., Hu, C. and Liu, L.F. (2007) Autophagy regulates ageing in *C. elegans*. Autophagy 3, 93–95.
[25] Tóth, M.L., Simon, P., Kovács, A.L. and Vellai, T. (2007) Influence of autophagy genes on ion-channel-dependent neuronal degeneration in *Caenorhabditis elegans*. J. Cell Sci. 120 (Pt 6), 1134–1141.
[26] Samara, C., Syntichaki, P. and Tavernarakis, N. (2008) Autophagy is required for necrotic cell death in *Caenorhabditis elegans*. Cell Death Differ. 15, 105–112.
[27] Conradt, B. and Xue, D. (2005) Programmed cell death. WormBook 6, 1–13.
[28] Levine, B., Sinha, S. and Kroemer, G. (2008) Bcl-2 family members: dual regulators of apoptosis and autophagy. Autophagy 4, 600–606.
[29] Takacs-Vellai, K., Vellai, T., Puoti, A., Passannante, M., Wicky, C., Streit, A., Kovacs, A.L. and Müller, F. (2005) Inactivation of the autophagy gene *bec-1* triggers apoptotic cell death in *C. elegans*. Curr. Biol. 15, 1513–1517.
[30] Qu, X., Zou, Z., Sun, Q., Luby-Phelps, K., Cheng, P., Hogan, R.N., Gilpin, C. and Levine, B. (2007) Autophagy gene-dependent clearance of apoptotic cells during embryonic development. Cell 128, 931–946.
[31] Mellén, M.A., de la Rosa, E.J. and Boya, P. (2008) The autophagic machinery is necessary for removal of cell corpses from the developing retinal neuroepithelium. Cell Death Differ. 15, 1279–1290.
[32] Morley, J.F., Brignull, H.R., Weyers, J.J. and Morimoto, R.I. (2002) The threshold for polyglutamine-expansion protein aggregation and cellular toxicity is dynamic and influenced by aging in *Caenorhabditis elegans*. PNAS 99, 10417–10422.
[33] Jia, K., Hart, A.C. and Levine, B. (2007) Autophagy genes protect against disease caused by polyglutamine expansion proteins in *Caenorhabditis elegans*. Autophagy 3, 21–25.
[34] Khan, L.A., Yamanaka, T. and Nukina, N. (2008) Genetic impairment of autophagy intensifies expanded polyglutamine toxicity in *Caenorhabditis elegans*. Biochem. Biophys. Res. Commun. 368, 729–735.
[35] Strome, S. (2005) Specification of the germ line. WormBook 1, 10.
[36] Hird, S.N., Paulsen, J.E. and Strome, S. (1996) Segregation of germ granules in living *Caenorhabditis elegans* embryos: cell-type-specific mechanisms for cytoplasmic localisation. Development 122, 1303–1312.
[37] DeRenzo, C., Reese, K.J. and Seydoux, G. (2003) Exclusion of germ plasm proteins from somatic lineages by cullin-dependent degradation. Nature 424, 685–689.
[38] Zhang, Y.X., Yan, L.B., Zhou, Z., Yang, P.G., Tian, E., Zhang, K., Zhao, Y., Li, Z.P., Song, B., Han, J.H., Miao, L. and Zhang, H. (2009) SEPA-1 mediates the specific recognition and degradation of P granule components by autophagy in *C. elegans*. Cell 136, 308–321.
[39] Kawasaki, I., Amiri, A., Fan, Y., Meyer, N., Dunkelbarger, S., Motohashi, T., Karashima, T., Bossinger, O. and Strome, S. (2004) The PGL family proteins associate with germ granules and function redundantly in *Caenorhabditis elegans* germline development. Genetics 167, 645–661.
[40] Kawasaki, I., Shim, Y.H., Kirchner, J., Kaminker, J., Wood, W.B. and Strome, S. (1998) PGL-1, a predicted RNA-binding component of germ granules, is essential for fertility in *C. elegans*. Cell 94, 635–645.
[41] Zhao, Y., Tian, E. and Zhang, H. (2009) Selective autophagic degradation of maternally-loaded germline P granule components in somatic cells during *C. elegans* embryogenesis. Autophagy 5, 717–719.
[42] Tsukamoto, S., Kuma, A., Murakami, M., Kishi, C., Yamamoto, A. and Mizushima, N. (2008) Autophagy is essential for preimplantation development of mouse embryos. Science 321, 117–120.
[43] Bjorkoy, G., Lamark, T., Brech, A., Outzen, H., Perander, M., Overvatn, A., Stenmark, H. and Johansen, T. (2005) P62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J. Cell Biol. 171, 603–614.
[44] Komatsu, M., Waguri, S., Koike, M., Sou, Y.S., Ueno, T., Hara, T., Mizushima, N., Iwata, J., Ezaki, J., Murata, S., et al. (2007) Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell 131, 1149–1163.
[45] Pankiv, S., Clausen, T.H., Lamark, T., Brech, A., Bruun, J.A., Outzen, H., Øvervatn, A., Bjørkøy, G. and Johansen, T. (2007) P62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J. Biol. Chem. 282, 24131–24145.
[46] Shintani, T., Huang, W.P., Stromhaug, P.E. and Klionsky, D.J. (2002) Mechanism of cargo selection in the cytoplasm to vacuole targeting pathway. Dev. Cell 3, 825–837.
[47] Ogura, K., Shirakawa, M., Barnes, T.M., Hekimi, S. and Ohshima, Y. (1997) The UNC-14 protein required for axonal elongation and guidance in *Caenorhabditis elegans* interacts with the serine/threonine kinase UNC-51. Genes Dev. 11, 1801–1811.

[48] Ogura, K., Wicky, C., Magnenat, L., Tobler, H., Mori, I., Müller, F. and Ohshima, Y. (1994) *Caenorhabditis elegans unc-51* gene required for axonal elongation encodes a novel serine/threonine kinase. Genes Dev. 8, 2389–2400.
[49] Lai, T. and Garriga, G. (2004) The conserved kinase UNC-51 acts with VAB-8 and UNC-14 to regulate axon outgrowth in *C. elegans*. Development 131, 5991–6000.
[50] Axe, E.L., Walker, S.A., Manifava, M., Chandra, P., Roderick, H.L., Habermann, A., Griffiths, G. and Ktistakis, N.T. (2008) Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J. Cell Biol. 182, 685–701.
[51] Hayashi-Nishino, M., Fujita, N., Noda, T., Yamaguchi, A., Yoshimori, T. and Yamamoto, A. (2009) A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat. Cell Biol. 11, 1433–1437.
[52] Eskelinen, E.L. (2005) Maturation of autophagic vacuoles in mammalian cells. Autophagy 1, 1–10.
[53] Fader, C.M. and Colombo, M.I. (2008) Autophagy and multivesicular bodies: two closely related partners. Cell Death Differ. 16, 70–78.
[54] Jahreiss, L., Menzies, F.M. and Rubinsztein, D.C. (2008) The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. Traffic 9, 574–587.